Glucose regulation of the IGF response system in chondrocytes: induction of an IGF-I-resistant state

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Kelley, K. M., T. R. Johnson, J. Ilan, and R. W. Moskowitz. Glucose regulation of the IGF response system in chondrocytes: induction of an IGF-I-resistant state. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1164–R1171, 1999.—Nonresponsiveness to the growth-stimulatory actions of insulin-like growth factor (IGF)-I in chondrocytes has been reported in a number of disease states associated with impaired glucose metabolism. Primary rabbit chondrocytes were investigated for changes in their IGF response system [type-I IGF receptor and IGF-binding protein (IGFBP) expression] and in their ability to mount a synthetic response to IGF-I [as [35S-labeled proteoglycan ([35S]PG) production] in media containing varying ambient glucose concentrations. Whereas basal [35S]PG synthetic rate was unaffected by glucose concentration, synthetic responsiveness to IGF-I was lost in media containing <5 mmol/l glucose or in media containing a “diabetic” glucose concentration (25 mmol/l). IGFBP expression, as measured by Northern analysis of mRNA levels and Western ligand blotting of secreted protein levels, was not significantly altered in the different glucose media, nor were there any differences in the cell surface localization of IGFBPs as assessed by affinity cross-linking with [125I]-labeled IGF-I, suggesting that IGFBPs do not induce the IGF-I resistance. The nonresponsiveness to IGF-I in reduced glucose occurred with 25–50% reductions in steady-state levels of IGF type-I receptor mRNA and protein. A significant correlation between IGF receptor mRNA level and synthetic response to IGF-I was observed between 0 and 10 mmol/l glucose concentrations, suggesting that the loss of responsiveness in reduced glucose is manifested at the level of transcription and/or receptor mRNA stability. In contrast, nonresponsiveness to IGF-I in chondrocytes in diabetic glucose concentrations occurred without changes in receptor mRNA and protein levels, suggesting that IGF-I resistance was due to post-ligand-binding receptor defects. It is proposed that IGF-I resistance in chondrocytes subjected to inappropriate glucose levels may constitute an important pathogenic mechanism in degenerative cartilage disorders.

IN ADDITION to its well-established mitogenic effects in chondrocytes and in a variety of cell types, insulin-like growth factor-I (IGF-I) plays an essential role in the promotion of differentiated cellular functions, which in chondrocytes occurs largely as the production of extracellular matrix (ECM) components (14, 16). IGF-I directly stimulates chondrocyte production of proteoglycan (PG), type-II collagen, and other ECM components (4, 6, 34). Indeed, the rate of chondrocyte [35S]PG synthesis (“sulfation activity”) has often been considered an adequate biochemical marker for the anabolic activity ascribed to IGF-I, so-called somatomedin activity (5). Although systemically available IGF-I is important for normal cartilage growth, chondrocytes themselves express the gene for IGF-I and synthesize and secrete IGF-I peptide (11, 16). Expression of the type-I IGF receptor and IGF-binding proteins (IGFBPs) has also been demonstrated in chondrocytes (16, 18, 25, 27), providing for a complete paracrine-autocrine IGF-IGFBP-IGF receptor regulatory axis. The dynamics of this essential endocrine axis in the regulation of chondrocyte growth and function are not well defined.

Degenerative cartilage diseases are characterized by a disequilibrium between ECM repair and degradative processes, with the former not keeping pace, resulting in a loss of PGs, collagen, and other ECM components (31). Increasingly, it has been recognized that a number of degenerative conditions in cartilage, as well as in other connective tissues, occur coincident with diseases associated with metabolic dysfunction (7). Several studies now indicate that reduced growth and repair in degenerative cartilage may be related to an inability of IGF-I to exert its effect on the chondrocyte target. For example, chondrocyte nonresponsiveness to IGF-I has been reported in a mouse model of inflammatory arthritis (31) as well as in patellar cartilage explants and articular chondrocytes from osteoarthritic humans (8). Kelley et al. (19) suggested that IGF-I resistance may be directly related to cellular metabolic impairment, after it was observed that cartilage explants of diabetic rats lacking glucose control were unresponsive to direct IGF-I treatment in vitro, while explants from normoglycemic diabetic rats showed a restored IGF-I response. In several additional studies, a strong association between growth impairment in cartilage and inhibited cellular glucose metabolism has been observed (22, 28, 30, 32, 33), although in these studies the effects on IGF-I action at the target level were not directly investigated.

The present study establishes that alterations in ambient glucose concentrations outside of normoglycemic ranges significantly impair the ability of IGF-I to activate PG synthesis in cultured rabbit chondrocytes, with apparently different mechanisms underlying the IGF-I resistance at high versus low glucose. Expression of the IGF type-I receptor and cell-produced IGFBPs was investigated in an attempt to identify the possible underlying endocrine mechanisms causing IGF-I resistance.
MATERIALS AND METHODS

Primary chondrocyte cultures. Female New Zealand White rabbits (Oryctolagus cuniculus; 2.0 ± 0.2 kg; Hazelton, Denver, PA) were euthanized in a CO2-flushed chamber, and their knee joints were removed and placed into sterile Gay's balanced salt solution (GBSS; Gibco-BRL, Grand Island, NY). All media were supplemented with 1% fungizone, 0.1% Nystatin, and 0.1% penicillin-streptomycin (Gibco-BRL). Cartilage was scraped from the articular surfaces of each knee and the shavings were placed into GBSS with 0.5 mg/ml hyaluronidase for 3 min. The shavings were then minced, placed onto a 45-μm precision woven screening mesh (Tetco, Elmsford, NY), and incubated for 30 min at 37°C in 2 mg/ml trypsin in GBSS. A final incubation in 2 mg/ml collagenase in GBSS for 90 min at 37°C freed the cells from the ECM, and the cells that cleared the mesh were collected, pelleted, resuspended in initial growth medium containing Ham's F-12, 10% fetal bovine serum (FBS; contains 6.5 mmol/l glucose), and antibiotics (all from Gibco-BRL). The cells were plated in six-well culture plates (Falcon, Oxnard, CA) at an initial density of 106 cells/ml. All experiments were carried out on confluent monolayers in serum-free (except where indicated) DMEM (Gibco-BRL), and different glucose concentrations were obtained by mixing appropriate volumes of DMEM lacking glucose with DMEM containing 25 mmol/l glucose.

Experiments. The effective dose range for IGF-I within the rabbit chondrocyte culture system was first assessed. Cells were placed into serum-free DMEM (10 mmol/l glucose) for 30 min (initial “wash”), followed by 24 h in the same medium with 2 μCi/ml [35S]SO4 and 0, 1, 10, 100, or 1,000 ng/ml human recombinant IGF-I (Genentech, San Francisco, CA). Synthesis of [35S]PG (Fig. 1A) was measured as described below. The same IGF-I dose response was tested in DMEM containing 5% FBS.

Confluent chondrocytes were then tested for their synthetic response to IGF-I over extended time periods in medium with or without 10 mmol/l glucose and/or 5% FBS. After a 30-min wash, as before, cells were cultured in DMEM containing 0 or 5% FBS and 10 or 0 mmol/l glucose. After preincubation periods of 1, 2, 4, and 7 days, cells were tested for their response to 100 ng/ml IGF-I (the most effective dose as defined above). Basal and IGF-I-stimulated [35S]PG synthetic activity was measured over 24 h in DMEM identical to that of the preincubation but with 2 μCi/ml [35S]SO4 with or without IGF-I.

The effect of five different glucose concentrations on synthetic response to IGF-I and on IGF type-I receptor mRNA levels was next examined. Confluent chondrocytes were washed as before and followed by a 48-h preincubation in serum-free DMEM containing 0, 1, 5, 10, or 25 mmol/l glucose. After each period, half of the cells (n = 3 wells/experiment) were harvested from a subset of the cells for subsequent RNase protection analyses for IGFBP-1–6 mRNA levels (described below). The remainder of the cells from each time point were incubated for an additional 24 h in identical media supplemented with 2 μCi/ml [35S]SO4 with (n = 3 wells/experiment) or without (n = 3 wells/experiment) 100 ng/ml IGF-I to measure cellular synthetic responsiveness to IGF-I.

Because IGFBPs are significant modulators of IGF action through their effects on the bioavailability and extracellular distribution of IGF peptides (18), the expression and possible cell surface localization of IGFBPs were then assessed. Confluent cells were washed and then cultured for 48 h in serum-free DMEM containing 0, 10, or 25 mmol/l glucose. After the cultures, conditioned medium was collected for measurement of secreted IGFBPs using a Western ligand blot procedure (described below), while the cell layer was subjected to an affinity cross-linking procedure (described below) to identify cell surface IGF-binding sites (receptors and/or any cell surface-associated IGFBPs). In addition, total RNA was harvested from a subset of the cells for subsequent Northern analyses for IGFBP-1–6 mRNA levels (described below).

Measurement of cellular [35S]PG synthesis. [35S]PG synthetic rate was measured during 24-h incubations in the presence of 2 μCi/ml [35S]SO4 (Amersham Life Sciences, Arlington Heights, IL), as described in Moskowitz et al. (26). Cell layers were precipitated in 10% TCA at 4°C for 15 min and then hydrolyzed overnight in 0.2 N NaOH at 37°C. Cell lysate aliquots were used to measure counts per minute (cpm) of [35S]PG and to determine protein concentration by the method of Lowry et al. (23). The medium was dialyzed against a 12,000 molecular weight cut-off membrane, and the resulting [35S]PG was counted. Total cpm from the cellular and medium components from each well were divided by total micrograms protein, and data were expressed as means ± SE of individual counts/min (cpm/μg) values (n = 8–10/treatment). Bars with different letter superscripts are significantly different from each other (P < 0.05).

Solution hybridization and RNase protection. IGF type-I receptor mRNA levels were measured by RNase protection using a 265-bp clone of the α-subunit region of the IGF type-I receptor as described by Werner et al. (35). Total RNA was quantified by absorbance at 260 nm using a Spectramax 250 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) and RNA quality was assessed by determining A260/A280 ratios (acceptable ratios were between 1.7 and 2.0) and by visual inspection of ethidium bromide-stained rRNA.
bands in 1.2% agarose gels. Antisense RNA probes were generated by transcription in the presence of guanosine 5'-[γ-32P]triphosphate (Amersham) from the SP6 promoter of a pGEM-3 plasmid containing the 265-bp receptor sequence. Ten micrograms total RNA were hybridized in solution to 400,000 cpm of antisense probe for 18 h and then digested with RNases A and T1 (GIBCO-BRL) for 60 min followed by a 30-min proteinase K (GIBCO-BRL) treatment. Each sample was then precipitated in ethanol using 10 µg yeast tRNA (GIBCO-BRL) as carrier, centrifuged, and the resulting pellet was washed twice in 75% ethanol, dried under vacuum, resuspended in 2% SDS, and counted (to obtain cpm of protected receptor mRNA). Blank controls (incubation of probe with 10 µg tRNA; n = 3 per experiment) were treated in identical fashion, and the resulting nonspecific cpm were subtracted from each sample total cpm value to obtain specific cpm. Data were normalized to micrograms total RNA (expressed as cpm/µg) and compiled from four repeated experiments. The samples were also run on 7% polyacrylamide-7 M urea gels followed by autoradiography to visualize the protected [32P]RNA bands.

Western ligand blot analysis. Conditioned medium samples from 48-h cultures of confluent chondrocytes were run into SDS-polyacrylamide gels (4% stacking, 12.5% separating gels) for 6 h at 20 mA/gel using a Hoeffer SE-400 slab gel apparatus (Hoeffer-Pharmacia, San Francisco, CA). The separated proteins were then transferred to nitrocellulose (NC) membranes using a Semi-Phor electrophoresis apparatus (Hoeffer), blocked in 3% gelatin in Tris-buffered saline (TBS; 50 mmol/l Tris, 0.9% NaCl, pH 7.5) for 6 h, and then incubated overnight in 3% gelatin in TBS containing 1,000,000 cpm/ml of 125I-labeled human IGFBP-1 (2.5 × 106 cpm/µg). IGF-I was iodinated with Na125I (Amersham) to a specific activity of 100–150 µCi/µg using a modified chloramine-T method. After incubation, membranes were washed several times in 0.1% Tween 20, then washed in 0.1% SDS, and counted (to obtain cpm of protected IGF-I) as carrier, centrifuged, and the resulting pellet was resuspended in 2% SDS, and counted (to obtain cpm of protected receptor RNA). Blanks controls (incubation of probe with 10 µg tRNA; n = 3 per experiment) were treated in identical fashion, and the resulting nonspecific cpm were subtracted from each sample total cpm value to obtain specific cpm. Data were normalized to micrograms total RNA (expressed as cpm/µg) and compiled from four repeated experiments. The samples were also run on 7% polyacrylamide-7 M urea gels followed by autoradiography to visualize the protected [32P]RNA bands.

RESULTS

At confluence, chondrocytes were tested for their anabolic responsiveness to IGF-I as measured by [35S]PG production during a 24-h incubation. Basal [35S]PG synthesis in serum-free DMEM approximated 25 cpm/µg, which was maximally stimulated to a level 2.7-fold greater in medium containing 100 ng/ml IGF-I (P < 0.01; Fig. 1A). Similarly, in DMEM containing 5% FBS, the presence of IGF-I significantly activated [35S]PG synthesis from basal levels. Basal [35S]PG synthesis in the presence of the serum was ~100 cpm/µg, with 100 ng/ml IGF-I resulting in a maximal 2.5-fold increase (P < 0.01; Fig. 1B). As 100 ng/ml IGF-I was found to be the most effective dose in stimulating [35S]PG synthesis under both conditions, this dose was used in subsequent tests of chondrocyte responsiveness to IGF-I.

The effects of excluding glucose over preculture periods of 1, 2, 4, and 7 days with or without 5% serum present were next tested on chondrocyte anabolic response to 100 ng/ml IGF-I. In DMEM containing a physiological glucose concentration (10 mmol/l) and supplemented with 5% FBS, [35S]PG synthesis was ~100 cpm/µg basally and was stimulated twofold in the presence of the IGF-I after all preculture periods (Fig. 2A). In serum-free DMEM (10 mmol/l glucose), the basal [35S]PG synthetic rate was significantly reduced (~25 cpm/µg); however, the chondrocytes nonetheless showed a twofold stimulation on IGF-I treatment (Fig. 2B). In contrast, when DMEM lacking glucose was used with (Fig. 2C) or without (Fig. 2D) 5% FBS supplementation, chondrocytes were rendered nonresponsive to IGF-I at all time points. Basal [35S]PG synthetic rate, however, was not significantly altered by differences in glucose concentration alone, nor did any of the cells show signs of detachment or nonviability (as assessed by trypan blue exclusion) throughout the 7-day experimental period.

Expression of IGF type-I receptor mRNA and response to IGF-I were next examined together in cells precultured in 0, 1, 5, 10, and 25 mmol/l glucose DMEM (serum free) for 48 h. As before, IGF-I (at 100 ng/ml)
was ineffective in stimulating \[^{35}S\]PG synthesis in medium lacking glucose (Fig. 3A). In medium containing 1 mmol/l glucose, \[^{35}S\]PG synthesis was also not altered by the IGF-I. In the physiological concentrations of glucose (5 and 10 mmol/l), however, IGF-I stimulated \[^{35}S\]PG by about twofold (P < 0.01). In medium containing a supraphysiological glucose concentration (25 mmol/l), the chondrocytes were again rendered unresponsive to IGF-I. IGF type-I receptor mRNA levels (Fig. 3B) were highest in the physiological glucose concentrations and decreased with increased glucose concentrations. The decrease in mRNA levels was significantly different from each other (P < 0.05). Receptor mRNA level was measured by solution hybridization/RNase protection. Data in B are expressed as means ± SE of cpm of protected \[^{32}P\]RNA per µg sample RNA (n = 12/treatment). Values with different letter superscripts are significantly different from each other (P < 0.05).
mRNA levels (Fig. 3) unresponsiveness did not occur with reduced receptor affinity. In contrast, in the 25 mmol/l glucose medium, chondrocyte unresponsiveness did not occur with reduced receptor mRNA levels (Fig. 3B). Figure 3C shows an autoradiograph of protected 32P-RNA bands in polyacrylamide-urea gels. As assessed by densitometry, the protected receptor RNA bands from chondrocytes cultured in 0, 1, 5, and 10 mmol/l glucose medium were 56.1 ± 6.7% reduced (P < 0.05) relative to bands for the 10 and 25 mmol/l glucose cultures. Affinity cross-linking of cell surface receptor protein (Fig. 6) corroborated these results (described below).

Because the lack in IGF-I responsiveness in 25 mmol/l glucose DMEM was not explained by reduced receptor mRNA levels, it needed to be determined whether there were any changes in levels of cell-secreted IGFBPs, as their presence could block IGF-I access to its cell surface receptor, inducing IGF-I resistance. As shown in Fig. 4, there were no discernable differences in the level of secreted IGFBPs among cells exposed to 25, 10, or 0 mmol/l glucose. The two IGFBPs secreted by the chondrocytes were identified as the 31-kDa IGFBP-5 and the 24-kDa IGFBP-4. Their mRNA levels and IGF-I responsiveness of [35S]PG synthesis (fold-stimulation) in chondrocytes in 0, 1, 5, and 10 mmol/l glucose were reduced by 23% (P < 0.05), whereas they were 50% lower in the 0 mmol/l glucose medium (P < 0.05) compared with levels in the physiological media. There was a significant positive correlation between IGF type-I receptor mRNA level and IGF-I responsiveness of [35S]PG synthesis (fold-stimulation) in chondrocytes in 0, 1, 5, and 10 mmol/l glucose (r = 0.7; P < 0.01; data not illustrated). In contrast, in the 25 mmol/l glucose medium, chondrocyte responsiveness did not occur with reduced receptor mRNA levels (Fig. 3B). Figure 3C shows an autoradiograph of protected 32P-RNA bands in polyacrylamide-urea gels. As assessed by densitometry, the protected receptor RNA bands from chondrocytes cultured in 0, 1, 5, and 10 mmol/l glucose medium were 56.1 ± 6.7% reduced (P < 0.05) relative to bands for the 10 and 25 mmol/l glucose cultures. Affinity cross-linking of cell surface receptor protein (Fig. 6) corroborated these results (described below).

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Finally, although IGFBP expression was unaltered, it was nonetheless conceivable that a shift in the distribution of local IGFBPs from the medium to cell surface could affect IGF-I interaction with its cellular receptor. Affinity cross-linking of the cell surface with 125I-labeled IGF-I (Fig. 6) demonstrated two specific binding sites, the 135-kDa IGF type-I receptor (IGF-binding) subunit and 31-kDa IGFBP-5 (located at ~38 kDa when covalently cross-linked with 125I-IGF-I). 125I-labeled bands at ~85 kDa were apparently nonspecific, because 125I-IGF-I binding to these bands could not be effectively inhibited by addition of excess unlabeled IGF-I peptide. Levels of the IGF type-I receptor α-subunit protein on the cell surface were not significantly altered in the 25 mmol/l group compared with the 10 mmol/l group, but were reduced by half in the 0 mmol/l glucose group, in support of the data shown in Fig. 3 for receptor mRNA levels. The 38-kDa band representing membrane-associated IGFBP-5 cross-linked to 125I-IGF-I was present at barely detectable levels on the cell surface and was not significantly altered between the 10 and 25 mmol/l glucose groups.

**DISCUSSION**

In the present study, it was demonstrated that chondrocyte synthetic response to IGF-I was inhibited when ambient glucose concentrations were outside of physiologically normal ranges. This lends support to the hypothesis that the coincidence of metabolic disorders with degenerative cartilage diseases (7, 28, 33) may be a result of a direct target effect of altered glucose metabolism. Previous in vitro studies of chondrocytes also suggest that changes in glucose concentra-
tion can directly affect cellular synthetic and growth functions. Leonard et al. (22) demonstrated that either low (−2.5 mmol/l) or high (−20 mmol/l) glucose concentrations can reduce [35S]PG production and PG core protein mRNA levels in avian precartilage mesenchymal cells. In mature rat mandibular arch chondrocytes, on the other hand, high glucose (44 mmol/l) reduced [3H]thymidine incorporation, whereas [35S]PG synthesis was not altered (32). Although the data are limited to date, these in vitro studies suggest that glucose may have important direct effects on the regulation of cartilage growth.

Glucose effects on cartilage growth were also suggested in a study on diabetic rats, in which costal cartilage explants from uncontrolled diabetic rats (−25 mmol/l serum glucose) were unresponsive to IGF-I in culture, whereas cartilage from normoglycemic diabetic rats (−6 mmol/l serum glucose) exhibited a strong [35S]PG synthetic response to added IGF-I (19). It was concluded that a state of IGF-I resistance in diabetic cartilage was the result of metabolic derangements associated with uncontrolled diabetes mellitus. The aim of the present study was to characterize the endocrine mechanism(s) that may underly glucose-regulated changes in cartilage synthetic activity, with regard to possible mediation by the IGF-IGFBP-IGF receptor axis.

In the present study, rabbit articular chondrocytes were responsive to addition of IGF-I under normal glucose conditions (5 and 10 mmol/l). Addition of IGF-I at 100 ng/ml, determined to be the most effective IGF-I dose, resulted in a twofold stimulation of [35S]PG synthesis, whether in the presence or absence of 5% FBS (Fig. 1). Removal of glucose from the medium, however, resulted in a loss of responsiveness to IGF-I. Although the absence of serum reduced basal levels of [35S]PG synthesis, it was only the exclusion of glucose that resulted in a loss of IGF-I response in the cells (Fig. 2). Glucose-deprived chondrocytes, although unresponsive, were viable as assessed by trypan blue exclusion. Furthermore, they synthesized [35S]PG at a basal rate similar to that of cells cultured in medium containing normophysiologic glucose (10 mmol/l), even out to 7 days of culture. Apparently, even in serum-free DMEM, the chondrocytes were able to use an alternative carbon source(s) in the glucose-deficient medium to remain viable and synthetically active at a basal level.

In addition to the exclusion of glucose, low glucose concentration (<5 mmol/l), as well as a diabetic glucose concentration (25 mmol/l), also resulted in an impaired chondrocyte response to IGF-I. The IGF-I resistance occurring in low glucose concentration was associated with reduced steady-state levels of IGF type-I receptor mRNA (Fig. 3). That the receptor’s protein levels were also decreased were evident in the affinity cross-linking data (Fig. 6), in which 125I-IGF-I-labeled 135-kDa α-subunit was also reduced by a similar magnitude (50%). There was a significant correlation between receptor mRNA levels and response to IGF-I at glucose concentrations between 0 and 10 mmol/l, suggesting that the effect of glucose restriction in causing IGF-I nonresponsiveness may be manifested at the level of transcription of the receptor gene and/or receptor mRNA stability. Although the effect on mRNA levels is likely to play an important role in this IGF-I resistance, it is also recognized that cells in reduced glucose may not generate enough ATP to support enhancement of anabolic processes; furthermore, signal transduction pathways of any remaining IGF receptors may also be impaired (see Ref. 15 and related discussion below).

Whereas low circulating glucose concentrations would not be expected in untreated insulin-dependent diabetics mellitus (IDDM), cellular glucopenia may occur in other diseases such as inflammatory arthritis. Peripheral insulin resistance, impaired glucose handling, and hyperinsulinemia have all been reported in rheumatoid arthritis (28, 33), in addition to significantly reduced synovial fluid glucose concentrations in both humans (21) and in a rabbit model (10). In synoviocytes isolated from patients with rheumatoid arthritis, glucose transport is insensitive to insulin action in vitro (13). Thus it is possible that under these disease situations, glucose deprivation leading to reduced type-I receptor expression and IGF-I resistance may constitute an important pathogenetic mechanism.

In the medium containing a glucose concentration similar to serum levels of glucose in uncontrolled diabetics, chondrocytes were again rendered unresponsive to IGF-I action (Fig. 3). Surprisingly, this IGF-I resistance occurred despite maintenance of IGF type-I receptor mRNA (Fig. 3) and protein (Fig. 6) at levels at least as high as those of IGF-I-responsive chondrocytes in physiological glucose. This result is similar to data on human osteoarthritic chondrocytes (8) and on diabetic rat cartilage (K. M. Kelley, unpublished data) in which normal to increased IGF type-I receptor expression occurred with IGF-I resistance. By analogy, growth hormone receptor levels are also not reduced in diabetic rats, but nonetheless a state of growth hormone resistance occurs as a result of postreceptor-binding defects (9, 24, 29).

It is also possible, however, that changes in locally expressed IGFBPs could alter chondrocyte response to IGF-I. Because IGFBPs possess high binding affinities for IGFs and significantly modulate IGF action in a variety of cell types (18), it was possible that increased chondrocyte secretion of IGFBPs in elevated glucose could cause IGF-I resistance by interfering with IGF-I interaction with its cellular IGF receptor. Several studies have now demonstrated IGFBP expression in chondrocytes (1, 8, 11, 25, 27). In the present study, of six possible IGFBPs examined, only IGFBP-4 and IGFBP-5 were detectable by Northern and Western ligand blot analyses, and neither IGFBP showed significantly altered expression (either mRNA or protein) with elevated medium glucose (Figs. 4 and 5). Thus, at least with regard to the expression of chondrocyte IGFBPs in culture, there is no evidence of glucose-induced changes in levels of IGFBPs that could serve to sequester and inhibit IGF-I peptide locally.

Despite the unchanged chondrocyte IGFBP expression, the possibility remained that a high glucose-
induced shift in the distribution of local IGFBPs to the cell surface could serve to inhibit IGF-I receptor binding. In a study of osteoarthritic human chondrocytes, it was reported that an IGFBP (believed to be IGFBP-3) was present on the surface of IGF-I-resistant cells in greater concentrations than on IGF-I-responsive control cells (8). In the present study, however, affinity cross-linking analyses indicated only slight cell surface localization of IGFBP-5 and no discernable changes in its distribution with changes in glucose concentration. We therefore conclude that the direct effects of changes in glucose concentration in inducing IGF-I resistance do not include mediation by the IGFBPs, whether soluble in the medium or on the cell surface; rather, we propose that the high glucose-induced IGF-I resistance may be related to IGF type-I receptor signal transduction defects.

In the closely related insulin receptor, signal transduction defects also appear to result from high glucose conditions. In a recent study by Hresko et al. (15), it was determined that insulin resistance in 3T3-L1 adipocytes, caused by glucosamine treatment, is tightly correlated with reduced intracellular ATP concentrations. Used to mimic high glucose-induced insulin resistance, glucosamine impaired several ATP-dependent aspects of insulin receptor transduction (e.g., receptor autophosphorylation, IRS-1 phosphorylation, PI 3 kinase activity) concomitantly with a loss in insulin-responsive glucose transporter activity (15). Earlier, Hawkins et al. (12) showed that IGF-I action, along with that of insulin, on glucose uptake and glycogen synthesis in rats was strongly inhibited in individuals treated with glucosamine. Together, these findings suggest that IGF receptor signal transduction pathways may be similarly impaired by high glucose and, furthermore, that IGF-I resistance could conceivably occur under any physiological condition that increases ambient glucose levels (for example, corticosterone-treated rats; see Ref. 30).

Although IGFBPs were not implicated in the present cell culture study, they nonetheless may still play an important role in tissue IGF-I resistance in vivo. Pronounced changes in circulating IGFBPs do occur with diabetes mellitus (see Ref. 18). IGFBP-1, in particular, is increased substantially in diabetic serum (20) and has been shown to inhibit IGF-I-mediated proliferation and [35S]PG synthesis when added to chick embryonic cartilage explants cultures (2). Because in the present study there was no evidence for IGFBP-1 gene expression in chondrocytes, such an inhibitory effect of this protein in vivo would require its delivery from the circulation to the chondrocyte target (a known property of IGFBP-1). Further studies are needed on IGFBP levels in diabetic or metabolically inhibited cartilage, in vitro and in vivo, to fully rule out the possible role of these regulatory proteins.

In summary, it was demonstrated that chondrocyte responsiveness to IGF-I was significantly impaired when ambient glucose concentration ventured outside normoglycemic ranges. Under conditions of glucose restriction, reduced IGF type-I receptor expression may account for reduced cell response to IGF-I. IGF-I resistance in high glucose, however, appears to be related to postreceptor-binding defects, because IGF receptor expression was not reduced under these conditions and there was no evidence for interference by locally expressed IGFBPs. That IGF-I is ineffective in promoting synthetic activity in chondrocytes subjected to altered ambient glucose may be an important pathogenic mechanism in cartilage degenerative diseases.

**Perspectives**

Clearly, expenditure of metabolic energy toward anabolic processes is maladaptive under conditions of poor fuel availability. The relationship between metabolism and growth is therefore a fundamentally important one with a number of "safeguards" in place that can shut down expensive growth functions under inappropriate metabolic circumstances. The mechanisms involved are represented in the changes in growth endocrine messengers and their receptors. It is now well established that in uncontrolled IDDM or in other metabolically impaired states, growth hormone-stimulated hepatic IGF-I production is blocked, a result of changes in the growth hormone receptor (see Refs. 5, 23, 29). In parallel, serum levels of IGFBPs also change dramatically and lead to inhibition of IGF-I action, as decreasing levels of growth-promoting IGFBP-3 occur with increases in levels of high-affinity IGFBP-1 that block IGF-I access to cellular IGF receptors (see Refs. 18, 20). These endocrine manifestations of the relationship between metabolism and growth are even apparent in representatives of earlier vertebrates (17).

In light of the present results, the relationship between metabolism and growth can be extended from the physiological/endocrine level to the more basic level of the cell itself. We showed that there is a direct relationship between ambient glucose levels and anabolic responsiveness to IGF-I, without the influence of "outside" endocrine factors (i.e., within a defined cell culture system), with IGF receptor expression (low glucose) or signal transduction (high glucose) apparently reduced in physiologically inappropriate ambient glucose concentrations. This sensitivity of the cellular IGF response system to metabolic factors has important implications to tissue degenerative pathologies. Perhaps even modest changes in glucose concentrations in the cellular microenvironment, such as those that can occur in poorly treated IDDM, could impair IGF-I-mediated anabolic activities, promoting a variety of cell and tissue complications.

The authors thank Beatta Boja for assistance in the chondrocyte culture work and also thank Salina A. Arpe and Prakash Desai (California State University at Long Beach) and Dr. Hemlata Pokharna and Susan Rudin (Case Western Reserve University) for help and/or advice.

These studies were supported by National Science Foundation Grant IBN-9600783 (to K. M. Kelley) and National Institute of Arthritis and Musculoskeletal and Skin Diseases Grants AR-30130 (to R. W. Moskowitz) and AR-20168 (to J. Ilan).

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